



IACUC GUIDELINE:	Rodent Husbandry and Breeding		
Guideline #	ACUP 502	IACUC Approval:	February 17, 2016

1. Introduction

This ACUP describes the basic procedures required for routine rodent care and breeding.

2. Materials

- A. Cage cards
- B. Feed
- C. Bedding
- D. Clean or sterilized water bottles & caps
- E. Clean or sterilized cages & lids
- F. Enrichment materials

3. Training

All students, faculty, and staff working with laboratory animals are required to complete the following training prior to any and all animal hands-on training:

a. Online training (CITI Program, www.citiprogram.org):

- i) Working with the IACUC, Basic Course
- i) **Protocol specific training-complete all courses related to the species:** Working with Mice in Research Setting, Basic Course
- ii) Working with Rats in Research Setting, Basic Course
- iii) Reducing Pain and Distress in Laboratory Mice and Rats, Basic Course
- iv) Aseptic Surgery, if applicable

b. Facility specific training:

- i) Animal facility orientation-introduction to animal facilities and laboratories where animal use occurs, including introduction to Animal Care and Use Standard Operating Procedures

c. Continuing Education:

- i) Annual submission of Health Assessment update
- ii) Triennial Refresher training

4. Occupational Health and Risk Assessment Program

All students, faculty, and staff will participate in the Occupation Health and Risk Assessment Program

5. Procedures

- A. Observation and care of animals
 - i) Observe all rodents daily (including weekends & holidays) for illness, injury, and general condition
 - ii) Provide routine care and husbandry for all rodents (including weekends & holidays)



- B. Emergency and holidays
 - i) Make provisions for emergency care by providing both contact information so that Principal Investigators (PI's) and their staff can be reached during and after business hours. Provide special requests or instructions as applicable.
- C. Animal identification methods
 - i) Identify all cages with a cage card. Include, at a minimum, the following information: species, strain, sex, number of animals in the cage, investigator and protocol number. If possible, date of birth or arrival should also be noted.
 - ii) Identify individual animals as needed in accordance with ACUP 509 Mouse Identification.
 - iii) Examples of individual identification methods include: ear punching, ear tags, micro-tattooing, or micro-chips
- D. Records and documentation
 - i) Refer to ACUP 513 for record keeping requirements at Texas A&M-Commerce animal facilities.
- E. Food and water
 - i) Feed animals to meet current National Research Council recommendations for rodent nutrition.
 - ii) Food storage
 - a. Store feed on pallets off the floor and at least 4 inches away from walls, in a vermin-proof storeroom.
 - b. Store open feed bags in leak-proof containers with tightly fitting lids.
 - c. Maintain temperature and humidity controls in the storeroom. Avoid temperatures above 70° F and extremes in humidity.
 - d. Use feed within 6 months of milling date.
 - e. Stack feed in a manner that allows easy reading of the milling date.
- iii) Water
 - a. Check water bottles daily for proper operation and cleanliness
 - b. If water level is lower than ½ full bottle may be refilled, ensuring that each bottle is returned to the same cage to prevent cross contamination.
 - c. Bottles should be replaced with clean ones when cages are changed, or sooner if any signs of contaminants are observed.
 - d. Monitor water quality routinely to ensure that it is free of contaminants that could potentially expose animals to chemical or infectious agents. Refer to Standard Operating Procedures for water quality monitoring requirements.
- F. Mouse breeding program
 - i. House breeders in either monogamous pairs or trio-breeding matings (i.e., one male and two females). Record dates of introductions.
 - ii. Remove the male from the breeding group either before females give birth, or shortly after birth to avoid more consecutive litters than desired per female. Record dates of birth and number of pups per litter.



- iii. In order to prevent overcrowding or to track pedigree lines, pregnant females in a trio mating group should be separated prior to giving birth; see section 7 of this document, Appendix-Mouse Trio Breeding Guidelines for further details.
- iv. Wean litters when next litter is born to prevent overcrowding in the cage. Record separation dates. Note that weaning prior to 19 days of age cannot be done without prior approval from the Attending Veterinarian.

G. Rat breeding program

- i. House breeders in monogamous pairs. Record dates of introductions.
- ii. Remove the male from the breeding group either before females give birth, or shortly after birth to avoid more consecutive litters than desired per female. Record dates of birth and number of pups per litter.
- iii. Wean litters when next litter is born to prevent overcrowding in the cage. Record separation dates.

H. Social and environmental enrichment

- i) Group house rodents, whenever possible
NOTE: Group housing, while an effective enrichment strategy, can sometimes lead to aggression and subsequent injury. Exercise caution when grouping unfamiliar animals, particularly with species or strains known to be aggressive, and allow extra time for observation.
- ii) Provide environmental enrichment devices for all rodents. Examples of enrichment include, but are not limited to, the following: PVC pipe, nest-lets, autoclaved blocks of wood, paper huts or Nylabones. Refer to ACUP 612 Rodent Enrichment for further information.

I. Quarantine Procedures

- i) Quarantine rodents from unapproved vendors for one to eight weeks depending on the status of both the vendor and the facility where they will be housed. The Attending Veterinarian will determine the quarantine requirements for each shipment.
Note: Quarantine of rodents from approved vendors is not required. An acclimation period of 7 days is strongly recommended for maximal adjustment. Experimental procedures conducted less than 72 hours after arrival must receive IACUC/veterinary approval. Quarantine period will be determined by the Attending Veterinarian.

J. Euthanasia and disposal of dead animals

- i) Euthanize rodents according to ACUP 301, conducted by trained staff only.
- ii) Dead animals may be stored in freezer until TAMU-C Waste Management pick-up dates.

Note: In special cases rodent carcasses are fed to snakes or other predatory animals. The carcasses utilized for food must be from healthy, non-transgenic rodents, which were euthanized with carbon dioxide. If frozen, containers must be clearly labeled regarding safety of food items.

K. Pest Control: refer to the Standard Operating Procedures, Vermin Program for a description of the vermin control program



L. Environment and Environmental Control

- i) Maintain room temperatures between 64 and 76° F. Refer to the Guide for species-specific temperature ranges
- ii) Maintain relative humidity between 30% and 70%
- iii) Maintain a daily log of min/ max room temperature and humidity.

M. Bedding

- i) Use wood chips, shredded paper products, or chopped corn cobs as bedding materials in solid-bottom cages
- ii) Use enough bedding to keep the rodents clean and dry until the next scheduled change

Note: Cedar shavings are not recommended due to the harmful presence of aromatic hydrocarbons

N. Lighting

- i) Provide a regular diurnal lighting cycle unless otherwise required by protocol
- Note:** Lights are controlled by timers set at a photoperiod of 12 to 14 hours of light. Check the timer performance routinely.

O. Space requirements

- i) Provide cages that are appropriate in size for the number of rodents housed in them. Refer to the recommendations set in Table 3.2 (pg. 57) of Guide for the Care and Use of Laboratory Animals

P. Cleaning of cages and water bottles

- i) Change cages and water bottles (if present) once every two weeks at a minimum
- ii) Change cages and water bottles (if present) more frequently if needed to keep the animals clean and dry and provide a healthy environment
- iii) Supply fresh bedding with each cage change with the exception of sentinel cages (refer to ACUP 607).

Q. Cleaning and sanitation of equipment

- i) Clean and sanitize equipment at least every two weeks, more frequently if needed

R. Transportation

- i) Transport rodents being shipped outside of Texas A&M-Commerce in filtered commercial rodent transport boxes
- ii) Transport rodents within Texas A&M-Commerce in either filter cages or unfiltered cages wrapped in a material which prevents animal allergens from escaping into the environment. Refer to ACUP 515

S. Waste Management

- i) Deposit non-regulated or non-infectious Medical Waste and soiled materials in dumpsters
- ii) Regulated (or infectious) Medical Waste is processed by Texas A&M-Commerce's Waste Management Facility.

6. Personnel Safety

- A. When handling animals, wear appropriate PPE, observe proper hygiene, and be aware of allergy, zoonosis, and injury risks. Refer to the TAMUC Occupational Health and Safety



webpage for more information.

B. Medical emergencies: Call 911

7. Animal Related Contingencies

- A. Post contact information for emergency assistance in a conspicuous location(s) within the animal facility
- B. Emergency veterinary care is available at all times including after working hours and on weekends and holidays
- C. Non-emergency veterinary questions & requests for care, contact the Attending Veterinarian at iacuc@tamuc.edu, or the Office for Research Compliance 903 886 5766

8. References

- A. *Guide for the Care and Use of Laboratory Animals, 8 Edition*. National Research Council; National Academy Press, Washington, DC, 2011.
http://www.nap.edu/openbook.php?record_id=12910
- B. *Laboratory Animal Management: Rodents*. National Research Council; National Academy Press, Washington, DC, 1996
- C. *Nutrient Requirements of Laboratory Animals, Fourth Revised Edition*, Subcommittee on Laboratory Animal Nutrition, Committee on Animal Nutrition, Board on Agriculture, National Research Council, 1995
- D. AVMA Guidelines on Euthanasia
2013 <https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>
- E. ACUP 612 Rodent Enrichment
- F. ACUP 301 Euthanasia of Laboratory Animals
- G. ACUP 509 Mouse Identification
- H. ACUP 513 Maintaining Health and Procedure Records

9. Appendix

Mouse Trio Breeding Guidelines

Appendix: Mouse Trio Breeding Guidelines

Background:

Trio mating is the preferred breeding method for breeding certain strains and lines of mice. Its benefits include:

- Better breeding performance
- More adult animals to assist with rearing of progeny and more females who may serve as foster mothers.
- Conservation of animal resources - one male can service two females.

However, trio breeding, when not managed properly, can lead to increased pup mortality, decreased reproductive efficiency, decreased pup growth rate or negatively altered parental or pup behavior. This is particularly true in the following cases:

- For strains or stocks that produce large litters
- When excessive waste may accumulate between routine static microisolator cage changes
- When disparities between litter ages put younger litters at risk due to marked



differences in pup activity

The following guidelines are designed to maximize the benefits of trio breeding. Any deviations from these guidelines, including use of a different cage type, should be described in the animal use protocol and approved by the IACUC before implementation.

Guidelines:

1. If the median litter size per female is (or expected to be) > 6 pups: the breeder female must be removed to a separate cage when identified as pregnant. The other female can stay with the male as a pair. This will prevent overcrowding of the cage.
2. Similarly, if trio is housed in standard (small) caging or to maintain clear parentage information, remove one female to a separate cage when identified as pregnant.
3. If the median total litter size (from both females) is >9 but <12: The male should be removed to a separate cage when pups are 14 days old.
4. If the median total litter size (from both females) is 9 or less: Trio breeding can continue without removal of either parent.

Because female mice can breed during post-partum estrus, a female may have suckling litter at the same time that they give birth to a new litter. The PI and/or lab staff must remain vigilant for this and wean the older (first) litter within 24 hrs of younger (second) litter birth but not before day 19.

In the rare event that a large litter is born unexpectedly (e.g., a litter of 10 is born to a female with median litter size of 5), it may not be necessary to separate the litter and mother, provided that there is no deleterious effect to the pups of either litter. This determination must be made in consultation with and approval by the Attending Veterinarian.

Litter size monitoring:

The Principal Investigator (PI), his/her research staff, or the breeding coordinator should maintain records on median litter size. This information provides the basis for guideline recommendations 1, 2, & 3 presented above. If any problems or concerns arise with litter size or separating mice, please contact the Attending Veterinarian.

Impact of differences in litter age on litter health:

Currently, there are no data on the impact of differences in litter age among pups that are co-housed. Researchers performing trio-breeding should monitor the litter health when there is significant (>5 days) disparity in age to ensure that the younger litters are not negatively affected.

References:

- Strategies for Maintaining Colonies of Laboratory Mice: A Jackson Laboratory Resource Manual Ray Lambert (Sr. Editor) 2009.
- Fox, W.M. Reflex-ontogeny and behavioural development of the mouse. *Animal Behaviour*. 1965; 13(2): 234-41.
- The Mouse in Biomedical Research, Fox JG, Barthold S, Davisson M, Newcomer CE, Quimby FW, Smith A 2nd edition 2006.



- Whitaker J, Moy SS, Saville BR, Godfrey V, Nielsen J, Bellinger D, Bradfield J. The Effect of Cage Size on Reproductive Performance and Behavior in C57BL/6 Mice. Lab Animal. 2007; 36(10): 32-39.

History:

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